PATENT SPECIFICATION

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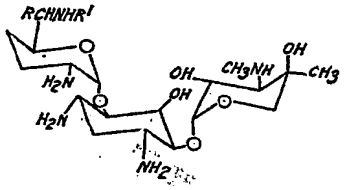
(19)

(54) NEW AMINOGLYCOSIDE ANTIBIOTICS

(71) We, MERCK & CO., INC., a corporation duly organized and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

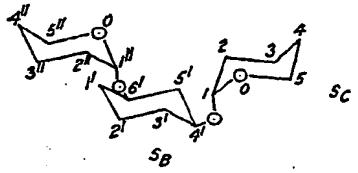
Gentamicin is an antibiotic substance produced by fermentation of *Micromonospora purpurea* or *M. echinospora* and variants thereof. It was first isolated and described in U.S. Patent 3,091,572 (1963). It is a highly effective antibiotic active against both gram-positive and gram-negative microorganisms such as species of Staphylococcus, Klebsiella, Pseudomonas, and Proteus. Three distinct but closely related chemical components have been separated and identified and are generally known as (gentamicin) C_1, C_2, or C_{1a}. (Another name for gentamicin C_{1a} accepted by the art is gentamicin D). The structure of these is as follows:



where in gentamicin C_1, R=R'=CH_3; in gentamicin C_2, R=CH_3 and R'=H; and in gentamicin C_{1a}, R=R'=H. The separation of these three components is described in U.S. Patent 3,651,042 (1972).

The nomenclature of the above can be simplified by recognizing that each portion of the molecule is numbered and named. For instance, the following scheme may be used: (the functional groups are removed to simplify the illustration)

II



The three rings are called S_A, S_B, and S_C, respectively, with the numbering as indicated. The S_B portion is also called the "deoxystreptamine" moiety. S_C is also called "garosamine"; while S_A and S_B together are called "gentamine".

Gentamicin has been recognized since its commercial introduction (in 1969) as a highly effective antibiotic of great value in the treatment of infections. As with many

[*Price 25p*]

antibiotics, however, extrachromosomal resistance to gentamicin can be induced *in vitro* by repeated sub-cultures in less than inhibitory concentrations of the antibiotic. Resistance to gentamicin has also been observed recently in the clinic. This chromosomal resistance is a relatively recently recognized phenomenon of bacteria, see Watanabe, T., (1963). "Infective heredity of multiple drug resistance in bacteria", *Bact. Rev.* 27: 87—115. The general phenomenon has been defined as "R-factors". Very generally, an R-factor is a biochemical capability of the bacteria to convert the antibiotic into a chemical derivative which does not interfere in the bacterial replication, thereby permitting bacterial growth. The clinical symptom of R-factor resistance is, of course, the failure of the patient to respond to the drug treatment. Gentamicin is still an active antibiotic, and as such continues to be useful in the war against disease. However, it is desirable to obtain a compound which has broad spectrum activity even against strains of organisms which exhibit resistance to gentamicin.

This invention provides derivatives of Gentamicin C_1, gentamicin C_{1a} or gentamicin C_2 having attached to the C—2 carbon of ring S_C a C_{1-6} alkoxy, carbamoyl, (C_{1-6} alkyl)carbamoyl, (C_{2-5} alkenyl)carbamoyl, oxo, epi-hydroxy, epi-methanesulfonyl, amino or epi-amino group or a hydrogen atom, the hydroxy and methanesulfonyloxy groups having the epi-steric position.

The invention also provides Gentamicin C_1, gentamicin C_{1a}, or gentamicin C_2, having on the C—5′ carbon of ring S_B a C_{1-6} alkoxy, carbamoyl, (C_{1-5} alkyl)carbamoyl, (C_{2-6} alkenyl)carbamoyl or methanesulfonyl substituent.

Further the invention provides Gentamicin C_1, gentamicin C_{1a} or gentamicin C_2 having two carbamoyl substituents on both the C—2 carbon of ring S_C and the C—5′ carbon of ring S_B.

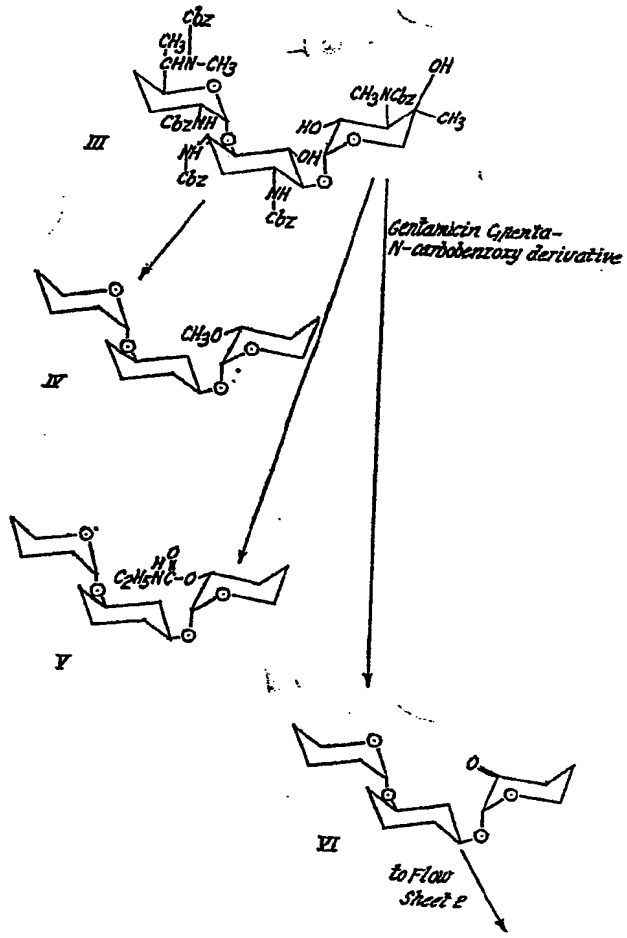
Compounds of the present invention have been found to exhibit a highly comprehensive activity spectrum against gram-positive and gram-negative infections, including certain organisms which are resistant to gentamicin. The individual derivatives do not necessarily have identical activity spectra, but each possesses activity as well as advantages over one or more of the parent gentamicins.

The nomenclature is trivial; if the new derivative has the same configuration at C—2 or C—5′ as the original hydroxyl group on the gentamicin, no prefix is used; however, if opposite configuration arises at C—2 or C—5′, the product is designated epi-. The nomenclature is more fully understandable with reference to the following flow sheets.

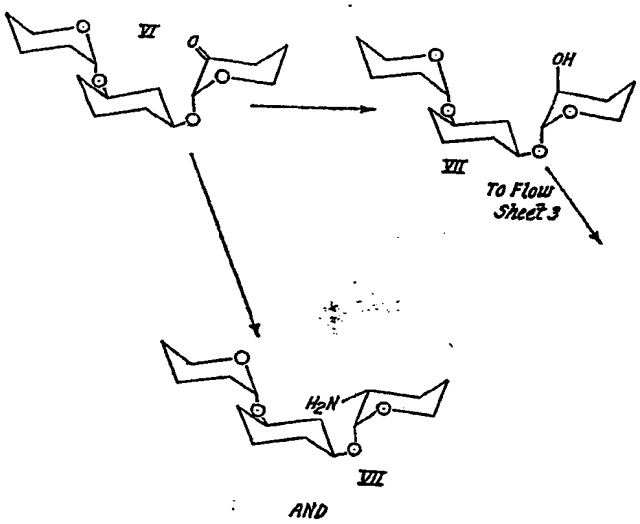
It is noted that the flow sheets use gentamicin C_1 to illustrate the reactions. It is intended that gentamicin C_2 and gentamicin C_{1a} (gentamicin C_D) be covered by the flow sheet also. In addition, the products IV—XIV all have the same structural substituents as the starting material, gentamicin C_1 penta-N-carbobenzoxy derivative except as indicated in the C—2 or C—5′ positions of the sugar rings S_C or S_B, respectively.

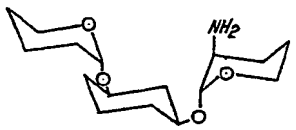
The compounds of the invention can be prepared using the following general processes. Reference can be made to the four Flow Sheets.

FLOW SHEET 1



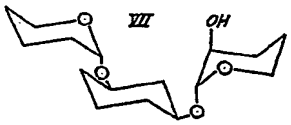
FLOW SHEET 2

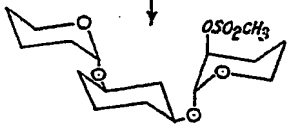




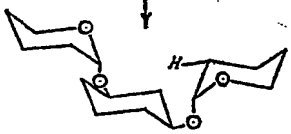
IX

FLOW SHEET 3

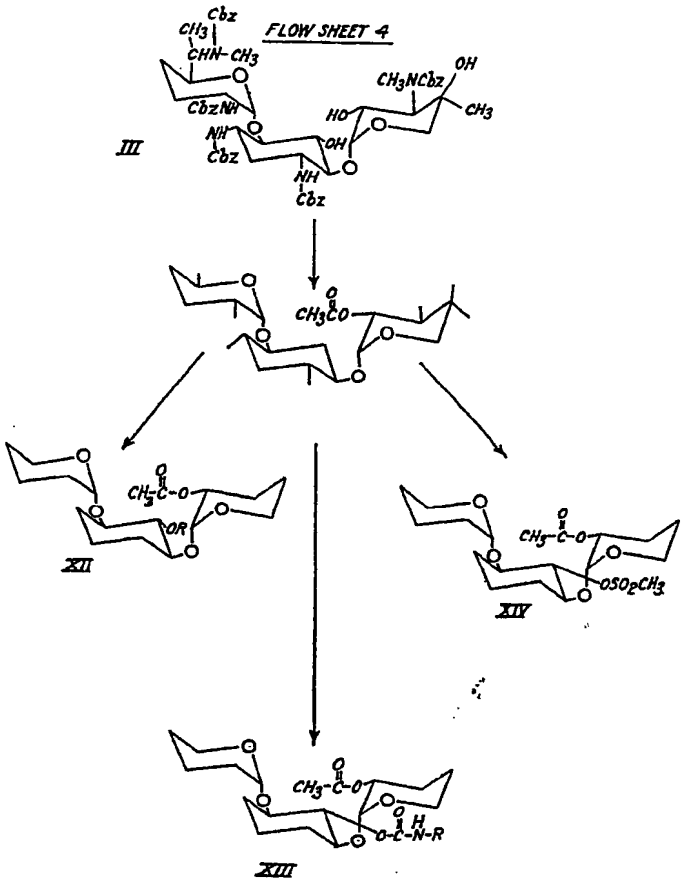




X



XI



The four flow sheets illustrate the general synthesis of the active compounds. The blocking groups can be removed as a last step to yield the gentamicin having the derived functional group at C—2 or C—5′.

The compounds of this invention can be prepared using the following reaction conditions.

The starting material for any of the reactions can be gentamicin C_1, C_2, or C_{1a}. In any case, the free amino groups are first blocked by reacting the desired component with a reagent capable of substituting an inert functionality onto the free hydrogens of the five amino groups. A suitable reagent is carbobenzoxy chloride; however, acetic anhydride can also be used. The gentamicin and the blocking reagent are stirred together, preferably at a temperature of −10°C. to 25°C. After purification and isolation using chromatographic techniques, the blocked gentamicin is recovered as a granular solid. Hereafter, the term "blocked gentamicin" will be used to refer to the key intermediate III, on which most reactions depend, but is understood to refer to each component of gentamicin C_1, C_2 and C_{1a}. Although the discussion herein refers to "active compounds", it is noted that this term refers to a final substituted gentamicin from which the blocking groups have been removed. The removal of blocking groups is the last reaction, involving a catalytic reduction using hydrogen over a 10% palladium/carbon catalyst, in the case of the carbobenzoxy group; or alkaline hydrolysis in the case of the acetyl group. In the latter case, either a catalytic amount of sodium methoxide in methanol or barium hydroxide in water can be used, or similar reagents.

The next general step in the sequences of Flow Sheets 1—3 involves the reaction at the unblocked C—2 hydroxyl. Although there are two other hydroxyl groups on the gentamicin, these do not participate in the reaction. The C—5 hydroxyl on S_B is sterically hindered by the S_A/S_C rings, while the C—4 hydroxyl on S_C, being tertiary, is not strongly reactive.

However, for the purposes of Flow Sheet 4, the C—2 hydroxy group is blocked by the formation of a C_{1-6} alkyl ester or C_{1-6} alkyl ether derivative. For example, an acetate group, using acetyl chloride or acetic anhydride, can be formed. As is evident, the C—5′ hydroxy group does not normally participate in the reactions outlined in Flow Sheets 1—3 due to the steric hindrance of the two S_A and S_O rings. However, if the C—2—OH is blocked and the reaction times increased five to fifty-fold, then the C—5—OH residue will react as desired. Preparative thin-layer chromatography will be used to separate and isolate the desired product. The two different types of blocking groups will then be removed, first the group of the C—2 hydroxyl, then the groups at the amino residues using standard techniques to yield the desired products. Therefore, the general description of the process is applicable to the C—5 hydroxyl modifications in Table II.

The 2-O-(C_{1-6} alkyl) group can be substituted on the blocked gentamicin by reacting with a C_{1-6} alkyl halide, preferably methyl iodide in an organic solvent. It may be desirable to use diazomethane to prepare the 2-O-methyl derivative. The reaction proceeds best under anhydrous conditions at a temperature of 25—50°C. for a reaction time of ½ to 12 hours. This group is illustrated as Compound IV in Flow Sheet I. The products are separated by chromatographic techniques. Not only is the 2-O-(C_{1-6} alkyl) compound formed by this procedure, but also the 2,5′-di-O-(C_{1-6} alkyl)-3,4,N_1O-carbonyl-tetracarbobenzoxy derivative. This latter product can be separated from the simple C_{1-6} alkyl compound and is also active as an antibacterial agent. As noted, this procedure is also applicable to the 5′-O-(C_{1-6} alkyl)-containing compounds XII.

The 2-O-(C_{1-6} alkyl)-carbamoyl or 2-O-(C_{2-6} alkenyl)-carbamoyl group, Compound V, Flow Sheet I, is prepared by the reaction of C_{1-6} alkyl isocyanate or C_{2-6} alkenyl isocyanate and the blocked gentamicin. The 2-O-carbamoyl group (also Compound V) is prepared by reaction of approximately equimolar amounts of trichloroethoxy carbonyl isocyanate and the blocked gentamicin. This results in the preparation of an intermediate compound having a trichloroethoxycarbonylcarbamoyl group at C—2. The blocking group can be removed with zinc dust in hot methanol to afford the desired 2-deoxy-2-carbamoyl group. If desired, reaction conditions can be adjusted to remove the blocking groups at the same time as the cleaving of the trichloroethoxycarbonyl group. The desired product is eluted using ethyl acetate in chloroform; 3/1 by volume on a silica gel plate. The fraction containing product is identified by its fluorescence under ultraviolet light. It is physically removed from the plate with a suitable organic solvent and obtained as a solid by evaporation of solvent. Both these procedures are applicable to the preparation of Compound XIII in the 5′-substituted series. The 2,5′-disubstituted carbamoyl compounds can also be prepared by increasing the length of the reaction time and doubling the quantity of trichloroethoxycarbonyl isocyanate. The course of reaction is monitored by thin-layer chromatography.

Compound VI, Flow Sheet I, is a valuable compound in its own right, and as it can be used to prepare other active compounds VII, VIII, IX, X, and XI, as described in Flow Sheets 2 and 3. This compound VI, the 2-keto derivative, is prepared by oxidizing the blocked gentamicin. Suitably, chromium trioxide-pyridine complex is used in an organic solvent such as methylene chloride. The oxidation proceeds rapidly at ambient temperatures. The residue, after solvent removal, is chromatographed and separated into fractions. The 2-keto fraction can then be purified.

The 2-amino and 2-epi-amino compounds, VIII and IX, Flow Sheet 2, are prepared by first reacting freshly prepared hydroxylamine with the 2-keto gentamicin VI. The 2-oximino intermediate thereby prepared is then reduced using a sodium metal reduction in anhydrous solvent. Both amino isomers are prepared and are separated and purified using preparative thin-layer chromatography.

The 2-epi-hydroxy derivative VII is useful *per se*, and also in preparing additional compounds X and XI in Flow Sheet 3. The 2-epi-hydroxy derivative itself is prepared from the 2-keto compound VI by sodium borohydride reduction in alcohol and dimethyl formamide. This reduction is not stereospecific, so that both the desired 2-epi-hydroxy gentamicin and the starting gentamicin are produced. The two isomers are separated by thin-layer chromatography, and the desired 2-epi-hydroxy compound isolated.

The 2-epi-methanesulfonyloxy derivative X is prepared from the 2-epi-hydroxy compound VII by reacting the latter with methanesulfonyl chloride in a solvent, such as pyridine. After a relatively brief reaction time, generally less than two days, the product is separated using preparative thin-layer chromatography.

The preparation of the 5′-methanesulfonyl derivative XIV uses similar reagents to the above procedure. It is first noted that the configuration of the 5′-substituent in Compound XIV does not change during this reaction; therefore, the term "epi" is not

used. The preparation of Compound XIV can be briefly described as follows: following preparation of the penta-N blocked and 2-blocked gentamicin, H is reacted in an inert solvent, such as pyridine, with a large excess of methane sulfonyl chloride. The reactants are then kept, operably at room temperature, for from 7—15 days to allow the reaction to proceed to completion. The reaction is monitored using thin-layer chromatography. Following isolation of the reaction product, the C—2 and the N-blocking groups are removed sequentially.

The 2-deoxy derivative XI is prepared by first reacting sodium benzyl mercaptide in ethanol at reflux with the 2-epi-methane sulfonyloxy compound X. The 2-benzylthio intermediate thereby prepared is then reduced, using Raney nickel and ethanol. The reaction mixture is stirred vigorously during reaction, generally from 2—15 hours. The reaction is monitored by subjecting samples to thin-layer chromatography during the course of reaction. When reaction is complete, the product, the 2-deoxy compound XI, is recovered using preparative thin-layer chromatography.

As was mentioned above, compounds IV to XI are blocked gentamicins. After removal of the blocking group by catalytic hydrogenation, the final products are isolated.

Compounds of this invention are useful as antibacterial agents. They possess broad-spectrum activity against diseases caused by species of *Proteus, Pseudomonas, Staphylococcus, Klebsiella,* and *Entrobacter,* particularly *Staphylococcus aureas, Klebsiella,* and *E. Coli.* For example, a chronic infection caused by *Proteus* can be efficaciously treated at a dose level of 1 mg./kg./body weight administered three times a day.

In addition, the compounds of this invention show antiviral activity against *Rickettsia akari.*

For administration to patients, the antibiotics are administered using normal procedures. Unit dosage forms can be prepared using pharmaceutically acceptable carriers. The compounds can be used as described above or in a salt form such as the sulfate. It is most desirable to administer the drugs in injection form; an injectable solution can be easily prepared using 50 g. of compound in sterile solution to a volume of 1.0 liter. The compounds can also be prepared for use in topical ointments or ophthalmic drops.

This invention is further illustrated by the following examples.

EXAMPLE 1

Gentamicin C_1 Penta-N-Carbobenzoxy Derivative

A mixture of gentamicin C_1 (954 mg.), sodium carbonate (1.06 g.), 12 ml. water and 36 ml. acetone is cooled to −5°C. Carbobenzoxy chloride (1.96 g.) is added with stirring during 5 minutes. The mixture is stirred in an ice bath for three hours and at 22°C. for one hour. The mixture is poured into 150 ml. cold water, and extracted with 2×25 ml. ethyl acetate. The ethyl acetate solution is dried over magnesium sulfate, concentrated to a glass, and triturated in hexane to give 2.10 g. (91%) of granular solid, m.p. 85—95°C.

Anal. Calcd. for $C_{61}M_{73}N_3O_{17}$: C, 63.81; M, 6.41; N, 6.10. Found: C, 63.92; M, 6.26; N, 5.93.

This procedure can also be used to yield the analogous gentamicin C_2 or C_{1a} derivatives.

EXAMPLE 2

Gentamicin C_{1a} Penta-N-Acetyl Derivative

A solution of 1 g. gentamicin C_{1a}, 20 ml. acetic anhydride, and 25 ml. methanol is stirred at 22°C. for 2.5 hours. The reaction solution is evaporated and the residue is chromatographed on Baker silica gel using chloroform and chloroform-methanol mixtures as eluants. The desired product, gentamicin C_{1a} penta-N-acetyl, is obtained as an amorphous solid, 1.15 g., 78% yield.

This procedure can also be carried out using gentamicin C_2 or gentamicin C_1.

EXAMPLE 3

Gentamicin C_1 Penta-N-Carbobenzoxy-2-O-Methyl Derivative

A mixture of 0.3 g. gentamicin C_1 penta-N-carbobenzoxy derivative, prepared as in Example 1, (or the analogous gentamicin C_2 or C_{1a} derivative), 0.6 g. anhydrous barium oxide, 0.9 ml. methyl iodide and 3 ml. dimethylformamide is stirred at 50°C. for 3 hours while protected with a Drierite tube. The mixture is filtered and the filtrate is lyophilized. The residue is chromatographed on 25 g. silica gel (Baker) using ethyl-acetate/chloroform; 3/1 by volume as eluant to afford the gentamicin C_1 penta-N-

carbobenzoxy-2-O-methyl derivative and the 2,5′-di-O-methyl-3,4-N,O-carbonyltetracarbobenzoxy derivative. In the same manner, the analogous C_{1-6} alkoxy derivatives can be prepared using the desired C_{1-6} alkyl halide.

The 2-O-methyl derivative can be also made by the use of the following process: A solution of 0.3 g. gentamicin C_1 penta-N-carbobenzoxy derivative, 30 ml. of dimethoxyethane and 10 ml. water is heated at 80°C. Diazomethane (~5 g.) in ethereal solution is added dropwise with vigorous stirring as the ether is allowed to distil from the reaction mixture. After completion of addition, the reaction mixture is evaporated under reduced pressure to remove most of the dimethoxyethane. The residue is extracted with chloroform, and the extract is dried with magnesium sulfate and evaporated. Chromatography on Baker silica gel using mixtures of chloroform and ethyl acetate separates the desired O-methyl derivative from unreacted starting material.

EXAMPLE 4

Gentamicin C_2 Penta-N-Carbobenzoxy-2-O-Methylcarbamoyl Derivative

A solution of 100 mg. gentamicin C_2 penta-N-carbobenzoxy derivative, prepared as in Example 1, 100 μl. methyl isocyanate, 6 μl. triethylamine and 3 ml. of chloroform is heated in a sealed tube at 60°C. for 3 days. The reaction mixture is evaporated under reduced pressure and the residue is placed on an 8″×8″ silica gel G chromatography plate using methylene chloride. The plate is eluted with ethyl-acetate/chloroform: 3/1 by volume. The area that fluoresces under ultraviolet light is scraped off and eluted with ethyl acetate. Evaporation affords gentamicin C_2 penta-N-carbobenzoxy-2-O-methylcarbamoyl derivative, 89 mg.

The analogous C_{1-6} alkyl carbamoyl derivatives can be prepared in a similar manner using the desired C_{1-6} alkyl isocyanate. Also, C_{2-6} alkenyl carbamates can be prepared, e.g., gentamicin C_2 penta-N-carbobenzoxy-2-O-allyl carbamate, using the reaction scheme above with allyl isocyanate as the reagent.

EXAMPLE 5

Gentamicin C_{1a} Penta-N-Carbobenzoxy-2-O-Trichloroethoxy-Carbonylcarbamoyl Derivative

To 200 mg. of gentamicin C_{1a} penta-N-carbobenzoxy derivative (prepared as in Example 1) in 4 ml. benzene is added 50 μl. of trichloroethoxycarbonyl isocyanate. After stirring at 22°C. for 2.5 hours, thin-layer chromatographic monitoring indicates reaction is about 2/3 complete. Addition of additional 10 μl. trichloroethoxycarbonyl isocyanate with stirring for 1.0 hour completes the reaction.

The reaction mixture is evaporated under reduced pressure and chromatographed on 25 g. Baker silica gel using ethyl-acetate/chloroform: 1/2 by volume as eluant. With collection of 50-ml. fractions the product is found in fractions 10 to 18. Combination of these fractions affords 127 mg. of the desired product, identified by elemental analysis as the gentamicin C_{1a} penta-N-carbobenzoxy-2-O-trichloroethoxycarbonylcarbamoyl derivative.

EXAMPLE 6

Gentamicin C_{1a} 2-Carbamoyl Derivative

A mixture of 80 mg. of gentamicin C_{1a} penta-N-carbobenzoxy-2-O-trichloroethoxycarbonylcarbamoyl derivative, 6 ml. dioxane, 4 ml. water, 5 drops acetic acid, and 80 mg. 10% palladium on charcoal is reduced catalytically at 22°C. and atmospheric pressure for 3 hours. The reaction mixture is filtered to remove catalyst, and the filtrate is lyophilized to afford 43 mg. of crude product. Preparative thin-layer chromatography on silica gel G with MeOH/$CHCl_3$/conc. ammonia: 2/1/1 by volume (lower phase) as eluant affords the product in 21 mg. yield. The product is identified by TLC, IR and mass spectrum as gentamicin C_{1a} 2-carbamoyl derivative.

EXAMPLE 7

Gentamicin C_{1a} 2,5′-Di-O-Carbamoyl Derivative

When the processes of Examples 5 and 6 are repeated, except that twice the quantity of trichloroethoxy carbonyl isocyanate is used in the reaction of Example 5, the major product isolated is the gentamicin C_{1a} penta-N-carbobenzoxy-2,5′-di-O-trichloroethoxycarbonylcarbamoyl derivative, 150 mg. When this latter is reduced using the process of Example 5, 70 mg. of the product, the gentamicin C_{1a} 2,5′-di-O-carbamoyl derivative, is obtained. Identity of the product is confirmed using infra-red and mass spectrophotometric analysis.

The gentamicin C_1 and gentamicin C_2 2,5′-di-O-carbamoyl derivatives can also be made using this process (yields, respectively, are 75 mg. and 70 mg.).

EXAMPLE 8

Gentamicin C_1 Penta-N-Carbobenzoxy-2-Keto Derivative

An oxidizing solution of 0.6 g. chromium trioxide, 0.97 ml. pyridine and 15 ml. of methylene chloride is prepared. To a solution of 0.3 g. of gentamicin C_1 penta-N-carbobenzoxy derivative in 5 ml. of methylene chloride is added 5 ml. of the oxidizing solution. After stirring for one hour, the mixture is filtered and the filtrate evaporated under reduced pressure. The residue is chromatographed on 25 g. of Baker silica gel using EtOAc/$CHCl_3$: 1/1 by volume as eluant. Evaporation of appropriate fractions affords gentamicin C_1 penta-N-carbobenzoxy-2-keto derivative.

EXAMPLE 9

Gentamicin C_1 Penta-N-Carbobenzoxy-2-epi Derivative

To a solution of 0.10 g. of gentamicin C_1 penta-N-carbobenzoxy-2-keto derivative in 1 ml. of N,N-dimethylformamide and 15 ml. of methanol is added sodium borohydride (100 mg. portion wise). After 2 hours at 22°C., the mixture is heated quickly to reflux and cooled. Evaporation under reduced pressure affords a residue to which 3 ml. of water is added. Extraction of this mixture with chloroform (4×5 ml.), drying of the chloroform with magnesium sulfate, and evaporation gives a residue. The residue is chromatographed on 25 g. silica gel G using ethyl-acetate/chloroform as eluant to give both gentamicin C_1 penta-N-carbobenzoxy derivative and its epimer at C—2.

EXAMPLE 10

Gentamicin C_1 Penta-N-Carbobenzoxy-2-Deoxy-2-Epi-Methane-Sulfonyloxy Derivative

A mixture of 0.1 g. gentamicin C_1 penta-N-carbobenzoxy-2-epi derivative, 40 ml. methanesulfonyl chloride and 2 ml. pyridine is stirred at 22°C. for 2 hours. The mixture is evaporated under reduced pressure and the residue is subjected to preparative thin-layer chromatography on an 8″×8″ silica gel G plate using ethyl-acetate/chloroform: 3/1 by volume as eluant. The area that fluoresces under ultraviolet light is scraped off and eluted with ethyl acetate, which on evaporation affords gentamicin C_1 penta-N-carbobenzoxy-2-deoxy-2-epi-methanesulfonyloxy derivative.

EXAMPLE 11

Gentamicin C_1 Penta-N-Carbobenzoxy-2-Deoxy Derivative

A solution of 0.1 g. gentamicin C_1 penta-N-carbobenzoxy-2-deoxy-2-epi-methanesulfonyloxy derivative, 0.1 g. sodium benzylmercaptide and 15 ml. of ethanol is refluxed for 10 hours. The solution is evaporated, and the residue is put on an 8″×8″ silica gel G thin-layer chromatography plate and developed with ethyl-acetate/chloroform: 3/1 by volume. The area that fluoresces under ultraviolet light is scraped off and eluted with ethyl acetate. Evaporation provides gentamicin C_1 penta-N-carbobenzoxy-2-deoxy-2-benzylthio derivative, which is not further characterized, but used directly in the next step.

A mixture of 0.1 g. gentamicin C_1 penta-N-carbobenzoxy-2-deoxy-2-benzylthio derivative, 2 g. of Raney nickel, and 15 ml. of ethanol is stirred vigorously at 22°C. Progress of the reaction is followed by thin-layer chromatography on silica gel G and ethyl-acetate/chloroform: 3/1 by volume as eluant. When reaction is complete, the mixture is filtered and the filtrate is evaporated under reduced pressure. The residue is subjected to preparative thin-layer chromatography on an 8″×8″ silica gel G thin-layer chromatography plate developed with ethyl-acetate/chloroform: 3/1 by volume. The area that fluoresces under ultraviolet light is scraped off and eluted with ethyl acetate. Evaporation provides gentamicin C_1 penta-N-carbobenzoxy-2-deoxy derivative.

EXAMPLE 12

Gentamicin C_1 Penta-N-Carbobenzoxy-2-Deoxy-2-Amino Derivative and Gentamicin C_1 Penta-N-Carbobenzoxy-2-Deoxy-2-Epi-Amino Derivative

A solution of 0.2 g. gentamicin C_1 penta-N-carbobenzoxy-2-keto derivative in 5 ml. ethanol is treated with one molar equivalent of freshly prepared hydroxylamine in ethanol. The solution is stirred for three hours at 22°C. and evaporated under reduced pressure. Treatment of the residue by preparative thin-layer chromatography as in Example 9 affords gentamicin C_1 penta-N-carbobenzoxy-2-deoxy-2-oximino derivative, which is not further characterized but used directly in the next step.

A solution of 0.2 g. gentamicin C_1 penta-N-carbobenzoxy-2-deoxy-2-oximino derivative in 10 ml. anhydrous ethanol is heated under reflux and 0.4 g. sodium is added gradually in small pieces during 15 minutes. The reaction mixture is cooled to room temperature and water is added cautiously. The mixture is evaporated under

reduced pressure and the residue is treated as in Example 9 by preparative thin-layer chromatography to afford gentamicin C_1 penta-N-carbobenzoxy-2-deoxy-2-amino derivative and gentamicin C_1 penta-N-carbobenzoxy-2-deoxy-2-epi-amino derivative.

EXAMPLE 13

Gentamicin C_{1a} Penta-N-Acetyl-2-O-Acetyl Derivative

A solution of 202 mg. of the gentamicin C_{1a} penta-N-acetyl derivative prepared as in Example 2 in 4 ml. pyridine containing 0.5 ml. acetic anhydride is kept at 22°C. for 2 days. The reaction mixture is then evaporated to dryness. The solid is chromatographed on silica gel using chloroform methanol mixtures as eluants to give 199 mg. of product, the gentamicin C_{1a} penta-N-acetyl-2-O-acetyl derivative, identified by IR and mass spectrophotometric techniques.

EXAMPLE 14

Gentamicin C_2 Penta-N-Carbobenzoxy-2-O-Acetyl Derivative

An ice-cold solution of penta-N-carbobenzoxy-gentamicin C_2 (5.10 g., 4.5 mmoles) prepared as in Example 1, in anhydrous pyridine (45 ml.) is treated with acetyl chloride (0.64 ml., 2 equiv.). The ice-bath is not replenished and the reaction mixture is stirred at room temperature for 16 hours.

Water (0.5 ml.) is added, and after being stirred for 1 hour at room temperature, the reaction mixture is evaporated to dryness *in vacuo*. Trituration of the residue with cold water affords the gentamicin C_2 penta-N-carbobenzoxy-2-O-acetyl derivative as a white, amorphous solid, homogeneous by TLC, R_f 0.39 ($CHCl_3$/EtOAc 1:1 v/v). The yield is quantitative.

EXAMPLE 15

Gentamicin C_{1a} Penta-N-Acetyl-2-O-Acetyl-5'-O-Methyl Derivative

To a solution of 84 mg. gentamicin C_{1a} penta-N-acetyl-2-O-acetyl derivative in 2 ml. dimethylformamide is added 15 ml. thallous ethoxide and 50 drops of methyl iodide. After 2 hours at 22°C., the sample is evaporated under reduced pressure to give 120 mg. of crude product, gentamicin C_{1a} penta-N-acetyl-2-O-acetyl-5'-O-methyl derivative.

The analogous C_{1-6} alkyl derivatives can be prepared in a similar manner by using the desired lower alkyl iodide or halide in the above procedure.

EXAMPLE 16

Gentamicin C_{1a} 5'-O-Methyl Derivative

The compound prepared in Example 15, the gentamicin C_{1a} penta-N-acetyl-2-O-acetyl-5'-O-methyl derivative (120 mg.), is mixed with 658 mg. barium hydroxide octahydrate and 4 ml. water, and heated in an oil bath at 100—110°C. for 32 hours and at 22°C. for 55 hours.

To the reaction mixture is added 0.1 ml. conc. sulfuric acid and the pH is adjusted to 2 with dilute sulfuric acid. The precipitate is removed by centrifugation and the filtrate is lyophilized to afford 110 mg. crude product. Preparative TLC on silica gel G using $CHCl_3$/MeOH/Conc. NH_3: 2/1/1 by volume (lower phase) as eluant affords 7.5 mg. of desired gentamicin C_{1a} 5'-O-methyl derivative, identified by mass spectroscopy and R_f value on TLC (above 2:1:1 by volume system, silica gel G, product has R of 0.27 compared to 0.23 for gentamicin C_{1a}).

The other 5-O'-(C_{1-6} alkyl) derivatives are analogously prepared.

EXAMPLE 17

Gentamicin C_2 Penta-N-Carbobenzoxy-2-O-Acetyl-5'-O-Carbamyl Derivative

A solution of gentamicin C_2 penta-N-carbobenzoxy-2-O-acetyl prepared as in Example 14 (3.53 g., 3.0 millimoles) in dry benzene (75 ml.) is treated with trichloroethoxycarbonyl isocyanate (0.47 ml., 15% molar excess) and the mixture is stirred at room temperature for 24 hours.

Excess of reagent is destroyed by addition of methanol (1 ml.), and the reaction mixture is evaporated to dryness *in vacuo*. The product (4.2 g.) is a yellow oil, by TLC a complex mixture, the major component of which is penta-N-carbobenzoxy-gentamicin C_2 2-O-acetyl-5'-O-trichlorocthoxycarbonyl carbamoyl, R_f 0.48 ($CHCl_3$/EtOAc 1:1 v/v), which is used directly in the next step.

Crude penta-N-carbobenzoxy gentamicin C_2 2-O-acetyl-5'-O-trichloroethoxycarbonyl carbamoyl (4.73 g., 3.4 mmoles) is dissolved in glacial acetic acid (200 ml.), zinc dust (18.9 g.) is added, and the mixture is stirred vigorously for 3 hours at room temperature.

The mixture is filtered, and the collected solid is washed with several portions of acetic acid. Evaporation of the combined filtrate and washings affords a colorless oil (*ca.* 5 g.). The major component of this oil is identified as penta-N-carbobenzoxy gentamicin C_2 2-O-acetyl-5'-O-carbamoyl, R_f 0.43 (EtOAc/$CHCl_3$ 3:1 v/v).

The major product is isolated by chromatography on a column of silica gel (250 g.) using ethyl-acetate/chloroform (3:1 v/v) as developer/eluant. Evaporation of appropriate fractions gives penta-N-carbobenzoxy gentamicin C_2 2-O-acetyl-5'-O-carbamoyl (2.04 g., 49%) as a colorless glassy solid.

EXAMPLE 18

Gentamicin C_2 Penta-N-Carbobenzoxy-5'-O-Carbamoyl Derivative

A small cube of sodium metal is dissolved in dry methanol (50 ml.), and to the solution is added penta-N-carbobenzoxy gentamicin C_2 2-O-acetyl-5-O-carbamoyl (1.97 g., 1.6 millimoles). After being allowed to stand for 2.5 hours at room temperature, the solution is evaporated carefully *in vacuo* to give a pale yellow foam (*ca.* 2 g.). The desired product, penta-N-carbobenzoxy gentamicin C_2 5'-O-carbamoyl, R_f 0.20 (ethyl-acetate/chloroform 3:1 v/v), is separated by chromatography on a silica gel (100 g.) column using EtOAc/$CHCl_3$ (3:1 v/v) as developer/eluant. The product (990 mg., 52%) is obtained as a colorless glassy solid by combination and evaporation of appropriate chromatographic fractions.

EXAMPLE 19

Gentamicin C_2 Penta-N-Carbobenzoxy-2-O-Acetyl-5'-O-Ethylcarbamoyl Derivative

A solution of 100 mg. gentamicin C_2 penta-N-carbobenzoxy-2-O-acetyl derivative, prepared as in Example 14, 100 ml. ethyl isocyanate, 6 μl. triethylamine, and 3 ml. of chloroform is heated in a sealed tube at 60°C. for 15 days. The mixture is evaporated under reduced pressure and the residue is placed on an 8"×8" silica gel G chromatography plate using methylene chloride. The plate is eluted with ethyl-acetate/chloroform: 3/1 by volume. The area that fluoresces under ultraviolet light is scraped off and eluted with ethylacetate. Evaporation affords gentamicin C_2 penta-N-carbobenzoxy-2-O-acetyl-5'-O-ethylcarbamoyl derivative. The analogous (C_{1-6} alkyl)carbamoyl derivatives can be prepared in a similar manner using the desired C_{1-6} alkyl isocyanate.

EXAMPLE 20

Gentamicin C_2 Penta-N-Carbobenzoxy-5'-O-Ethylcarbamoyl Derivative

A small cube of sodium metal is dissolved in dry methanol (50 ml.), and to the solution is added penta-N-carbobenzoxy gentamicin C_2 2-O-acetyl-5'-O-ethylcarbamoyl (1.5 g). After being allowed to stand for 2.5 hours at room temperature, the solution is evaporated carefully *in vacuo* to give a pale yellow foam. The desired product, penta-N-carbobenzoxy gentamicin C_2 5'-O-ethylcarbamoyl is separated by chromatography on a silica gel (100 g.) column being EtOAc/$CHCl_3$ (3:1 v/v) as developer/eluant. The product, gentamicin C_2 penta-N-carbobenzoxy-5'-O-ethylcarbamoyl derivative, is obtained as a non-crystalline solid by combination and evaporation of appropriate chromatographic fractions.

EXAMPLE 21

Gentamicin C_2-Penta-N-Carbobenzoxy-2-O-Acetyl-5'-O-Methanesulfonyl Derivative

A mixture of 0.1 g. of gentamicin C_2 penta-N-carbobenzoxy-2-O-acetyl derivative prepared as in Example 14 is dissolved in 2 ml. pyridine containing 50 ml. methanesulfonyl chloride and stirred at room temperature. The reaction is allowed to proceed for 10 days. Monitoring of the reaction during this period by TLC indicates that the reaction is completed. The mixture is then evaporated under reduced pressure and the residue is subjected to preparative thin-layer chromatography on an 8"×8" silica gel G plate using ethyl-acetate/chloroform: 3/1 as eluant. The area that fluoresces under ultraviolet light is scraped off and eluted with ethyl acetate, which on evaporation affords gentamicin C_2 penta-N-carbobenzoxy-2-O-acetyl-5'-O-methanesulfonyl derivative.

EXAMPLE 22

Gentamicin C_2 Penta-N-Carbobenzoxy-5'-O-Methanesulfonyl Derivative

Following the same general procedure as that described in Example 20, gentamicin C_2 penta-N-carbobenzoxy-2-O-acetyl-5'-methanesulfonyloxy derivative is treated with

sodium in methanol. The product, gentamicin penta-N-carbobenzoxy-5-methanesulfonyl derivative, is separated using preparative thin-layer chromatography.

EXAMPLE 23

Removal of N-Carbobenzoxy Blocking Groups

The compounds prepared in Examples 3, 4, 8, 9, 10, 11, 12, 18, 20, and 22 are deblocked to remove the penta-N-carbobenzoxy groups. The following procedure is used; for illustrative purposes, the compounds of Examples 4 and 18 are mentioned specifically.

A. Gentamicin C_2 2-O-Methylcarbamoyl Derivative

A mixture of 45 mg. gentamicin C_2 penta-N-carbobenzoxy-2-O-methylcarbamate, 45 mg. 10% palladium on carbon, 4 ml. dioxane, 3.5 ml. water, and 2 drops acetic acid are hydrogenated at 22°C. and one atmosphere for 3 hours. The catalyst is removed by filtration and the residue is lyophilized to give 24 mg. crude product. The crude product is purified by preparative TLC on silica gel using $CHCl_3$/MeOH/Conc. NH_3: 2/1/1 by volume (lower phase) as eluant. The purified product weighed 13 mg. and is identified by mass spectroscopy as gentamicin C_{22} 2-O-methylcarbamoyl derivative.

B. Gentamicin C_2 5'-O-carbamoyl Derivative

Penta-N-carbobenzoxy gentamicin C_2 5-O-carbamoyl (615 mg., 0.52 mmole) is dissolved in a mixture of dioxane (15 ml.), water (12 ml.) and glacial acetic acid (10 drops), 10% palladium-on-charcoal catalyst (600 mg.) is added, and the mixture is hydrogenated at room temperature and atmospheric pressure for 3 hours.

The reaction mixture is filtered, and the filtrate is evaporated *in vacuo* affording a pale yellow oil (375 mg.). The crude product is chromatographed on a silica gel (70 g.) column using the lower phase of a $CHCl_3$/MeOH/conc. NH_4OH (2:1:1 v/v) solvent system as eluant. Evaporation of the appropriate chromatographic fractions gives gentamicin C_2 5'-O-carbamoyl (145 mg., 55%) as a white amorphous solid, homogeneous by TLC, R_f 0.15 ($CHCl_3$/MeOH/NH_4OH 2:1:1 v/v—lower phase).

The following compounds can be prepared using the above procedures:

gentamicin C_1 2-O-methyl
gentamicin C_{1a} 2-O-ethyl
gentamicin C_2 2-O-isopropyl
gentamicin C_1 2-O-ethylcarbamoyl
gentamicin C_{1a} 2-O-*n*-allylcarbamoyl
gentamicin C_2 2-O-hexylcarbamoyl
gentamicin C_2 2-O-isopropylcarbamoyl
gentamicin C_2 2-O-ethylcarbamoyl
gentamicin C_2 2-O-methylcarbamoyl
gentamicin C_1 2-keto
gentamicin C_{1a} 2-keto
gentamicin C_2 2-keto
gentamicin C_1 2-epi
gentamicin C_{1a} 2-epi
gentamicin C_1 2-deoxy-2-epi-methanesulfonyloxy
gentamicin C_2 2-deoxy-2-epi-methanesulfonyloxy
gentamicin C_1 2-deoxy
gentamicin C_{1a} 2-deoxy
gentamicin C_2 2-deoxy
gentamicin C_1 2-deoxy-2-amino
gentamicin C_{1a} 2-deoxy-2-amino
gentamicin C_2 2-deoxy-2-amino
gentamicin C_1 2-deoxy-2-epi-amino
gentamicin C_{1a} 2-deoxy-2-epi-amino
gentamicin C_2 2-deoxy-2-epi-amino
gentamicin C_2 5'-O-carbamoyl
gentamicin C_1 5'-O-carbamoyl
gentamicin C_{1a} 5'-O-carbamoyl
gentamicin C_2 5'-O-ethylcarbamoyl
gentamicin C_{1a} 5'-O-methylcarbamoyl
gentamicin C_1 5'-O-methylcarbamoyl
gentamicin C_2 5'-O-methanesulfonyl
gentamicin C_1 5'-O-methanesulfonyl

WHAT WE CLAIM IS:—

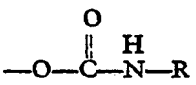
1. Gentamicin C_1, gentamicin C_{1a} or gentamicin C_2, having attached to the C—2 carbon of ring S_C a C_{1-6} alkoxy, carbamoyl, (C_{1-6} alkyl)carbamoyl, (C_{2-6} alkenyl)-carbamoyl, oxo, epi-hydroxy, epi-methanesulfonyl, amino or epi-amino group or a hydrogen atom, the hydroxy and methanesulfonyloxy group having the epi-steric position.

2. A compound as claimed in claim 1 in which the C—2 substituent is C_{1-6} alkoxy.

3. A compound as claimed in claim 2 in which the C—2 substituent is methoxy.

4. A compound as claimed in claim 2 in which the C—2 substituent is ethoxy.

5. A compound as claimed in claim 1 in which the C—2 substituent is



where R is hydrogen, C_{1-6} alkyl or C_{2-6} alkenyl.

6. A compound as claimed in claim 5 in which R is hydrogen.

7. A compound as claimed in claim 5 in which R is methyl.

8. A compound as claimed in claim 5 in which R is ethyl.

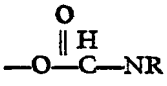
9. A compound as claimed in claim 5 in which R is isopropyl.

10. A compound as claimed in claim 5 in which R is allyl.

11. Gentamicin C_1, gentamicin C_{1a} or gentamicin C_2 having on the C—5′ carbon of ring S_B a C_{1-6} alkoxy, carbamoyl, (C_{1-6} alkyl)carbamoyl, (C_{2-6} alkenyl)carbamoyl or methanesulfonyl substituent.

12. A compound as claimed in claim 11 in which the substituent is methoxy.

13. A compound as claimed in claim 11 in which the substituent at C—5′ is



where R is hydrogen, C_{1-6} alkyl or C_{2-6} alkenyl.

14. A compound as claimed in claim 13 in which R is hydrogen.

15. A compound as claimed in claim 13 in which R is methyl.

16. A compound as claimed in claim 13 in which R is ethyl.

17. Gentamicin C_1, gentamicin C_{1a}, or gentamicin C_2, having two carbamoyl substituents on both the C—2 carbon of ring S_C and the C—5′ carbon of ring S_B.

18. A method of preparing a compound as claimed in claim 1, 11 or 17, that comprises treating a penta-N-blocked gentamicin having the desired C—2, C—5′ or di C—2, C—5′ substituent(s) under conditions to remove the blocking agent.

19. A method as claimed in claim 18 in which the penta-N-blocked gentamicin is blocked with either a carbobenzoxy or an acetyl group.

20. A method as claimed in claim 19 in which the carbobenzoxy blocking group is removed using hydrogen over a 10% palladium/carbon catalyst.

21. A method as claimed in claim 19 in which the acetyl blocking group is removed by alkaline hydrolysis.

22. A method as claimed in claim 21 in which the alkaline hydrolysis is effected using a catalytic amount of sodium methoxide in methanol.

23. A method as claimed in claim 21 in which the alkaline hydrolysis is effected using barium hydroxide in water.

24. A method as claimed in any one of claims 18—23, in which the penta-N-blocked starting material is prepared by a method substantially as hereinbefore described with reference to Flow Sheet I, II, III or IV.

25. A method as claimed in claim 24, in which the penta-N-blocked starting material is prepared by a method substantially as hereinbefore described with reference to any appropriate Example.

26. A method as claimed in claim 18, substantially as hereinbefore described in any appropriate Example.

27. A compound as claimed in claim 1, 11 or 17, when prepared by a method as claimed in any one of claims 18—26.

28. A pharmaceutically acceptable salt of a compound as claimed in any one of claims 1—17 and 27.

29. The sulphate of a compound as claimed in any one of claims 1—17 and 27.

30. A pharmaceutical composition containing, as active ingredient, at least one

compound as claimed in any one of claims 1—17 and 27—29, together with a pharmaceutically acceptable diluent or carrier.

31. A composition as claimed in claim 30 in injectable form.

32. A composition as claimed in claim 31 containing 50 g of the said compound to every litre of a sterile solution.

33. A composition as claimed in claim 30, in topically administrable form.

34. A composition as claimed in claim 33 in the form of an ointment.

35. A composition as claimed in claim 33 in the form of opthalmic drops.

36. A composition as claimed in claim 33 in the form of a cream or lotion.

37. A composition as claimed in claim 30 in the form of a pill, tablet, capsule, powder, granules, syrup or elixir.

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